UNIVERSITY OF ALBERTA

THE PRODUCTION OF MUTATIONS BY THE IRRADIATION OF MONTCALM BARLEY

A DISSERTATION

SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

FACULTY OF AGRICULTURE

DEPARTMENT OF PLANT SCIENCE

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EDMONTON, ALBERTA

APRIL, 1955

ABSTRACT

Five radiation sources were used to induce mutations in barley. All treatments were given at a dosage of 10,000 r equivalent. The radiation sources with their respective dose-rates in the region of the irradiated seeds were: a betatron (181.8 r/min.), an X-ray machine (201 r/min.), radium-beryllium (5.3 r/min.), and two Co^{60} sources (4.5 r/min. and 75.75 r/min.).

None of the radiation sources used was more effective than the X-ray treatment in producing mutations. The betatron and the high dose-rate treatments from Co^{60} appear to be somewhat less effective than X-rays.

Over 30 different mutant types were produced, including a number of vital mutants, such as stiff-strawed and early-maturing types. These appear promising as new varieties, but require further agronomic evaluation. It is concluded that mutation induction will become a useful new approach for plant breeders.

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I INTRODUCTION

Plant breeding work in many self-pollinated crops has been so exhaustive in utilizing readily available, plant material that further varietal improvements may, as far as conventional methods are concerned, be achieved in only two ways: First, by the addition of one or two valuable characteristics, such as disease resistance, to a suitable existing variety; or second by bringing new genetic variation into hybrid combination by new interspecific crosses. In other words, breeding work in the more important cereal crops is running rather low in new germplasm, the basic raw material.

This situation has created a need for finding new sources of variation. Since this is becoming increasingly difficult to do by convential methods, the production of mutations by irradiation may provide much needed and readily available new variability for utilization in the further improvement of self-pollinated crops.

Research in this field appears to have been somewhat discouraged in North America by Stadler's (44) disparagement of induced mutations as a source of plant breeding material. The present work was stimulated by a Swedish report (20) on X-ray induced vital mutants in barley which have possibilities as new commercial varieties or as good parental materials.

The objectives of the present project were fourfold:

(a) To produce agronomically useful mutants of Montcalm barley.

(b) To evaluate the findings of Swedish workers and to confirm results previously obtained at the University of Saskatchewan.

(c) To obtain a comparison of the mutagenic value of various radiation sources.

(d) To make at least a preliminary evaluation of induced mutation as a new technique in plant breeding.

II PHYSICAL NATURE AND PROPERTIES OF RADIATIONS

The types of radiations which have been found to cause mutations may be divided into two groups: electromagnetic radiations and corpuscular radiations (26). X-rays and gamma rays are examples of electromagnetic radiations. Neutrons are an example of corpuscular radiations.

Electromagnetic radiation is believed to consist of discrete energy quanta called photons with which is associated a wave length. All photons travel with the same speed in empty space. This speed known as c or the speed of light, is approximately 186,000 miles per second. The wave lengths (λ) of electromagnetic radiations vary over a wide range from long radio waves having a wave length of 1 to 2 miles to gamma rays having a wave length of about 10^{-9} to 10^{-12} cm. The wave length and velocity may be associated by means of the formula $F = c/\lambda$, where F is frequency. The energy of a photon or quantum is given by

the formula E = hF, where h is Planck's constant and is equal to 6.6×10^{-27} erg sec. (24)

Electromagnetic radiation may be produced by the following process: an electron may be raised from a lower to a higher energy level to give an excited atom. Subsequently the electron vacancy may be filled by any one of the electrons of higher energy, the vacancy so formed being filled by another electron of still higher energy, and so on until the atom has returned to the unexcited state. Each electron transition results in the emission of a photon and the sum of all the photon energies is equal to the original excitation energy.

Somewhat similar phenomena take place within the nuclei. Like an atom, a nucleus may be excited, in which case one or more of the nucleons is raised above the ground (or stable) state. A nucleus may be excited under bombardment by photons and nuclear particles. A nucleon may be ejected out of the nucleus. An excited nucleus returns to ground state by a series of particle and photon emissions. Nuclear photons are called gamma rays whereas atomic photons, previously discussed, are called X-rays. The phenomenon involving changes of unstable nuclei moving in the direction of stability is known as radioactivity.

Prior to gamma emmission in radioactive decay, particles such as alpha or beta are given off. In the cases where the particle carries off all the energy difference between the ground states of the parent and daughter nuclei, no gamma rays are emitted. In the decay of many isotopes however, the nucleus is left in an excited state

following particle emission and one or more gamma rays are emitted.

The energy of the particle type of radiation may be obtained by the formula $E = mc^2$, where E is particle energy, m is particle mass, and c is the speed of light.

The density of ionization of a particular type of radiation is the property in which geneticists are most interested. It is believed (5) that genetic changes are produced through ionization in the chromosomes and genes, and for this reason one may expect the more highly-ionizing radiations to be more effective in producing mutations (4, 30).

There is a considerable and growing body of evidence supporting the theory that genes may be large, nucleo-protein molecules, and that mutation may be in the nature of highly specific chemical changes in the molecule structure. For example, a flower colour mutant has been shown to be due to a gene change that causes substitution of OH for H at a specified position in the anthocyanin (pigment) molecule. Since ionization produces momentary chemical reactivity, it follows that it may produce chemical change in the molecule-gene.

Ehrenberg and Nybom (5) report using protons, (the chief ionizing particle in the case of neutron irradiation) so fast that they lead to about the same ion density as X-rays. They have demonstrated by growth inhibition experiments that the two radiations have approximately the same biological efficiency. They then concluded that the ion density produced is the only property of the

ionizing particle which is of importance in biological effectivity. These workers put forth the hypothesis "that the densely ionizing radiations act more directly on hereditary material than do the others which, in addition to more indirect genetical effects, also exert a damaging action on extranuclear cell constituents."

III LITERATURE REVIEW

A. General

Muller (35), working with Drosophila and Stadler (45), working with barley, were the first to report on the biological effects of radiations. Both reported that mutations could be produced artifically by irradiation of biological materials. This opened up a new field for biologists, especially geneticists and plant breeders.

Swedish workers have reported irradiation-induced mutations of various physiological and morphological types (11, 12, 14, 15, 16, 19, 20). Nybom (40) in a recent publication gives a summary of the various mutations which Swedish workers have obtained from irradiation studies. These studies show that radiations induce a wide range of mutations, ranging from very slight, almost undetectable changes to drastic, easily-observed morphological changes, and from lethal, economically-useless mutations to valuable new mutations which can be used as new varieties.

B. Induced Mutations and Plant Breeding

Opinions expressed in the literature concerning the usefulness

of radiation-induced mutations as a method or tool in plant breeding may be arbitrarily divided into two general categories, favorable and unfavorable.

Plant breeders not in favor of the use of induced mutations base their objections on the premise that characteristics of value in breeding are more likely to be found in a varietal collection since variability in that state is more likely to occur without the many undesirable gene mutations and chromosome aberrations frequently found in irradiated progenies. Those of a favorable mind assume the burden of proof, and base their opinions mainly on experimental evidence such as that presented in this paper.

Stadler (44) who first induced mutations in plants by irradiation did not believe that this technique offered much as a tool in plant breeding. His objections were essentially those outlined above. Stadler reported further that before induced mutations could be of use to plant breeding a method of selective induction would have to be found.

He does, however, suggest two possible uses for induced mutations in plant breeding. First, in inbred lines of corn induced mutations may be used to produce variability so that selection can be carried on further. Secondly, and with some enthusiasm, he suggests the use of induced mutations in fruit-tree breeding. Most of these trees are highly heterozygous and vegetatively reproduced for commercial purposes. Their long reproductive cycle makes breeding by

hybridization and subsequent selection a long, tedious, and often unrewarding job. But by induced mutations a new variation may be directly created which, because it may be vegetatively propagated, avoids the complications related to sexual reproduction.

Gustafsson (12, 15) reports that the plant breeder cannot neglect artificially induced mutants in the further improvement of his varieties. He points out that mutants must be evaluated on their merits as new varieties or as parental material.

Gustafsson and Tedin (21) report that the process of mutation can to some extent be controlled and directed by artificial means. Such a control is also suggested by Nilan (37). Gustafsson and Tedin (21) believe that mutation research is indespensable for the progress of plant breeding.

Oltman (41) predicted that by X-irradiation of plants it would be possible to obtain characters and qualities which have not yet been discovered in naturally variable material. Moreover, he reports that by induced mutations variations may be produced in crop material which has been thoroughly investigated by breeding and in which only small improvements can be produced by ordinary methods of breeding.

Several workers have obtained disease-resistant mutants by irradiation of plants. Ulonska (47) and Hoffman (25) report the induction of a mildew resistant form of spring barley, otherwise identical with the original variety. Similarly, Bandlow (2) reports finding a mildew resistant form of winter barley, apparently the first time that such resistance has been reported in this crop.

Tolbert and Pearson (46) reported that X-ray treatment of the groundnut has resulted in mutants with an increased range of variability in resistance to leaf spot. Konzak (28) reported that rust resistant mutants were obtained in oats after exposure to radioactive materials. He believes that mutants of agronomic value are to be found in irradiated populations. MacKey (17) has obtained a series of rust resistant mutants in wheat.

Stiff-strawed mutants in barley have been reported by several workers (11, 15, 20, 25, 38, 40, 42). They have also been obtained in wheat (33).

Gustafsson (15) reports obtaining three stiff-strawed mutants in barley at least equal in yield to the parent varieties. Similar results were obtained at Saskatoon (42).

Reports of other induced mutants of agronomic value are numerous. Increased earliness (15, 20, 25, 29, 38), increased drought resistance (18, 25), superior baking quality (25), and a wider range in protein content (range of parent 10-11 %, range of mutant 7-16 %) have been reported (25).

Swedish workers have distributed two X-ray-induced varieties, one in white mustard, "Primex", which produces 7 per cent more oil per hectare than the mother strain , and one in peas, "Stralart", which has a seed yield of 5 per cent more than the original variety (1, 8).

Several workers agree with Stadler on the possibilities of induced mutations in trees and shrubs. Granhall _et_ _al_. (9) and Granhall (10) report that results from X-rayed apple trees are very

encouraging from a plant breeding point of view. Bishop (3) suggests that X-rays may be used as an artificial method of inducing colour gene mutations in apples. Lewis (32) suggests the use of X-rays to produce dwarf plants, which may be used as precocious root stocks in fruit tree breeding, especially in the case of cherries and pears where the need has not been met by natural material. He also suggests that X-rays may be used to produce self-compatible plants in species which are normally self-incompatible.

Hagberg and Nybom (22) report two instances of X-ray induced mutations in potatoes.

Julen (27) reporting on X-ray treatment of Poa pratensis obtained one mutant that seems to be better than the normal plants.

As late as 1950 we have an adverse opinion from Hagedoorn (23), a Dutch geneticist, who appears very dubious about the use of induced mutations in plant breeding.

On the whole there appears to be a great deal of controversy over the degree to which induced mutations may prove useful in a plant breeding program. However, the favorable experimental evidence appears to be very substantial.

C. Studies Conducted on Barley

Much of the irradiation work conducted on barley has been based on treatments of the seeds.

A number of workers (6, 13, 31, 36, 37, 39, 43) report on various modifying factors and characteristics of the plants which alter the reaction of the treated seeds to irradiation. Six-rowed varieties

are reported to be more sensitive to X-rays than two rowed varieties (13). Larger seeds show greater germinability after X-ray treatment than do smaller seeds. Also the hull of barley and oats make hulled varieties less susceptible to radiation damage than hulless varieties (6). Several reports indicate that susceptibility to X-ray damage decreases with an increase in polyploidy (31, 36, 43). A recent Swedish report (33) suggests that a higher observable mutation frequency may be obtained by irradiation of a polyploid than by irradiation of a diploid because of the better tolerance by the polyploid of chromosomal disturbances.

Gelin (7) reported on the effects of increased moisture content on susceptibility of the seeds to X-ray damage. He found that an increase in mutation rate was achieved by increasing the moisture content of the seeds to be irradiated.

IV MATERIALS AND METHODS

Montcalm, the barley variety used in this study, is a blue-seeded, six-rowed, smooth-awned type with a moderately strong straw and high malting quality. It is susceptible to loose smut and leaf rusts, but semi-resistant to covered smut. It is one of the most widely grown varieties of malting barley in western Canada.

The radiation sources with their respective dose-rates in the region of the irradiated seeds were: an X-ray machine operated at 200 K.V.P. and 20.5 m.a. with no filter and a dose-rate of 201 r per minute; a betatron operating at 24 M.E.V. with a dose-rate of 181.8 r

per minute, a radium-beryllium source with a dose-rate of 5.3 r per minute, and two Co^{60} sources, one with a dose-rate of 75.75 r per minute (Co^{60} high), the other with a dose-rate of 4.5 r per minute (Co^{60} low). All treatments were made at 10,000 r.

A special mechanical device was developed to adapt the radium-beryllium source to treatment of seeds. The device consists of a central plastic cylinder, containing the source, surrounded radially by a number of small plastic cylinders in which the seeds are placed for treatment. Each of the seed containers revolves individually, and they all revolve as a group around the central cylinder containing the source. The device enables the treatment to be done with very little trouble. The operator has only to put the seed in the seed cylinders, put the source in the central cylinder, turn the device on and leave it for the desired length of time. The time will depend on the dosage desired.

The seed was treated and the X_1 grown at the University of Saskatchewan in the summer of 1952. In the fall of 1952 the material was harvested and taken to the University of Alberta. The number of X_1 plants harvested for further study and the treatments used are given in Table 1.

Table I. Details of treatments

X_1 plants, no.	Treatment	r/min.	Length of treatment, minutes
500	10,000r Co^{60} high	75.75	133.20
500	10,000r Co^{60} low	4.5	2221.80
500	10,000r X-ray	201.0	49.75
500	10,000r betatron	181.8	55.00
300	10,000r radium-beryllium	5.3	1875.00
500	checks		

In 1953 a maximum of five tiller-progenies from each X_1 plant were grown at Edmonton. The resulting X_2 population consisted of approximately 10,000 tiller-progenies. A check (Montcalm) was grown in every tenth row throughout the entire nursery.

In 1954 an X_3 generation, comprising most of the mutants found in the X_2, was grown at Swift Current, Saskatchewan. This population consisted of approximately 5000 progeny rows.

In the present investigation, as in most plant breeding projects on induced mutations, the population handled was too large to permit the use of special techniques for the detection of less obvious mutations. The range in mutations reported herein is thus limited to readily observable types.

V EXPERIMENTAL RESULTS

A. Observed Effects of Treatments in X_1

The irradiation treatments were made on lots of 1000 seeds

each during the period April 3-7, 1952. This seed was stored until May 2, at which time it was sown. Seedling emergence was very poor. Consequently additional seeds were treated during the period, June 2-6, and were sown in the field directly after treatment. The emergence from the latter treatments was good. Data on number of seeds treated and their emergence are given in Table II.

Only one lot of seeds was treated with radium-beryllium due to the unavailability of the source at the time of the second treatment.

The results indicate that storage of seeds after treatment may reduce their ability to survive irradiation treatments. Similar findings are also reported by Gustafsson (15) and Stadler (44).

Table II. Data on number of seeds subjected to each treatment and on number of seedlings subsequently emerged.

Lot no.	Treatment	Seeds treated, no.	Seedlings emerged, no.	Date treated
1	Co^{60} (high)	1000	57	April 5
2	Co^{60} (high)	2612	1098	June 2
1	Co^{60} (low)	1000	55	April 5-7
2	Co^{60} (low)	3035	1496	June 3-6
1	X-ray	1000	95	April 5
2	X-ray	2850	1442	June 4
1	betatron	1000	103	April 3
2	betatron	2424	1383	June 5
1	radium-beryllium	1000	343	May 6-14

Several twin heads were observed in the X_1. Seed from these plants gave rise to normal-appearing progeny.

Some sterility was observed in the late-sown X_1 material but, since it could not be determined with certainty whether this effect was due to the treatment or due to frost damage, no attempt was made to estimate the degree of sterility.

B. Observed Effects of Treatments in X_2

The X_2 in irradiation work may be compared with the F_2 of an ordinary cross for in both cases segregation occurs. In the X_2 the mutants which are very preponderantly recessive, segregate out and become visible, much as in F_2 segregation for recessive phenotypes.

For purposes of description the mutants found in the X_2 were divided into three general classes as follows:

1. Chlorophyll mutants.
2. Stiff-strawed mutants.
3. Other mutants.

A description of the mutants found, with their behavior in the X_3 (when that generation was grown) follows:

1. Chlorophyll mutants

The most readily detectable mutants observed in the X_2 were the chlorophyll-deficiency types, most of which were lethal, dying when the food supply was exhausted in the endosperm. Invariably these mutants appeared from only one tiller-progeny of an X_1 plant.

(a) Albinos

The albinos were pure white seedlings, being the most frequently-occurring of all the mutants observed in the project. The

albino character was lethal. The progeny of plants heterozygous for this character segregated in a ratio of 3 green to 1 white.

(b) Yellows

These mutants were similar to the albinos in that the character was lethal and in that the progeny from heterozygous plants segregated in a 3:1 ratio. They had a colour varying from light yellow to a golden yellow and were quite frequent in occurrence.

(c) Orange

The orange mutants were similar to the yellows except for their colour, which was an orange or reddish yellow shade. They were rare in occurrence, lethal and the progeny from heterozygous plants segregated in a 3:1 ratio of green to orange seedlings. This type of mutant was only noted in the X-ray treated material.

(d) Yellow-green

These plants, about equal to the yellow mutants in occurrence, were somewhat more yellowish than the normal seedlings, and gradually became greener until they were indistinguishable from the normal at the mature-plant stage. The progeny of heterozygous plants segregated in a ratio of 3 green to 1 yellow-green and homozygous plants breed true, as indicated by X_3 results.

(e) White, green-tipped

These mutants are very similar to the albinos except that the tips of the leaves are green. Of compartively rare occurrence, they died soon after emergence. The progeny of heterozygous plants segregated in a 3:1 ratio.

(f) Yellow, Green-tipped

These plants are identical with type (e) described above, except that they were yellow where type (e) were white. They were of rare occurrence, none being found in the progeny from the seeds treated with X-rays. The progeny of plants heterozygous for this mutant segregated in a 3:1 ratio. In most cases this character was lethal in the seedling stage, however, one or two plants survived to the mature-plant stage but failed to set seed.

(g) Variegated or Striped

Variegated plants, for the most part white plants with green stripes, were found in all treatments except Co^{60} low. These plants usually survived until maturity but due to a slower growth rate they were late and were frozen before ripening. The progeny from heterozygous plants segregated in a 3:1 ratio of green to variegated.

In addition to the variegated plants described above green plants with yellow striping were found in the betatron and Co^{60} high treatments.

(h) Mottled

Several plants were observed in the radium-beryllium treatment which were green with yellow spots, giving a mottled effect. The yellowish spots gradually became greener until at the mature-plant stage this mutant was indistinguishable from the normal. Plants heterozygous for the mottled character segregated in a 3:1 ratio.

(i) Grandpa

These plants were found in the radium-beryllium and

Co^{60} low treatments. Their leaves varied from considerable to very little variegation with white. The leaf sheath and culm were invariably white. The heads, awns, and aleurone were all white. This mutant was similar to one described by Martini and Harlan (34).

2. <u>Stiff-strawed Mutants</u>

Several plant progenies observed in the treatments were found to have considerably stronger straw than the adjacent Montcalm checks. Two distinct types of mutants which can be classified as stiff-strawed were observed.

(a) Stiff-strawed

This mutant was designated as stiff-strawed, for want of a more suitable name. These plants seemed to be stronger throughout the entire culm. They were distinguished on erectness of the heads that was in sharp contrast to the Montcalm plants which tend to have a nodding head. They seem to have an especially strong neck. This type of mutation was found in all treatments but Co^{60} high and bred true in the X_3. These mutants may turn out to be the most useful mutant as far as increased straw strength is concerned. A similar mutant obtained at Saskatoon (42) has shown up well in yields tests and appears to be equal to Montcalm in yield and superior to it in straw strength.

(b) Dense Headed

Mutants similar to this type were designated as erectoides by Swedish workers.

Although all plants in this group had dense heads, the

density varied somewhat from one line to another. The heads were usually shorter than those of Montcalm and the kernels slightly smaller. The heads were held very erect indicating a strong straw which was also evidenced by their good resistance to lodging. In all cases there were a number of observable characteristics associated with the dense head, suggesting that this character may be pleiotropic. This is supported by a report of Gustafsson (15) in which he states that erectoides are pleiotropic.

The progeny of plants heterozygous for the mutant segregated in a ratio of 3 normal to 1 dense, and homozygous plants bred true as indicated by X_3 results. Dense headed mutants were found in all treatments but Co^{60} low. The dense, compact type of head is shown in Fig. 1, nos. 2, 3 and 4.

Fig. 1. 1, Montcalm; 2, 3, 4, Dense headed; 5, Hooded; 6, Singed.

(c) Weak-strawed

These mutants showed a definite weakness of the stem and were lodged consistantly while the adjacent Montcalm checks were quite erect. This type was noted only in the betatron treatment.

3. Other Mutants

(a) Early Maturing Mutants

The mutants for earliness headed and matured from a week to ten days earlier than the checks. They were found in all five treatments, with the majority being observed in the Co^{60} low and the radium-beryllium treatments. These mutants are of much practical interest because of the need for early varieties in northern agricultural regions.

(b) Hooded Mutants

Three hooded plants were observed in one X_2 tiller progeny row of an X_1 plant from the X-ray treatment. (see Fig. 1, No. 5 and Fig. 2)

The hood differed considerably from that of Warrior (a standard hooded variety). It was considerably broader than that of Warrior and terminated in a short curly awn. In most cases there was an opening in the top of the floret and the florets were sterile. The few seeds obtained in the X_2 bred true for the hooded character in X_3. The progeny of plants heterozygous for the character segregated in a 3:1 ratio of normal to hooded.

(c) Singed

A whole X_2 tiller progeny row of an X_1 plant from the betatron treatment showed a mutant character designated as singed. (see Fig. 1, No. 6) The mutant appeared normal except for the awns extended only a short distance beyond the top of the head, if at all, and their tips were curled. The singed character was also noted in the X_3.

Fig. 2. Hooded

(d) Leafless

This mutant appeared in the X_2 from seed treated with

X-rays. It was characterized by a drastic reduction in size of leaves or, in some cases, a complete absence of leaves. (See Fig. 3). This character was also obeerved in the X_3 but the reduction in leaf area was not as drastic suggesting that the environment may influence its expression

Fig. 3 Left, Leafless; Right, Montcalm.

(e) Spindly or Vine-like

These plants occurred in the X_2 of seeds treated with X-rays. The plants were long, thin, vine-like, prostrate, and light green in color (See Fig. 4). The heads were lax, poorly developed, and sterile. Plants heterozygous for this mutant segregated in a ratio of 3 normal to 1 spindly.

Fig. 4 Spindly, or Vine-like, Mutant

(f) Tweaky

One X_2 tiller progeny row from seeds treated with the Co^{60} low source showed tweaky plants. These plants have heads with several florets missing at one or more locations on the rachis, thus leaving a bare space. The tweakiness was not very strongly expressed in the X_3, indicating that its expression may be influenced by

environment.

(g) Short Awns

One mutant appeared, in the X_2 of the Co^{60} high treatment, which had very fine, smooth awns. The awns of this mutant were also much shorter than the normal, extending about one-half as far beyond the head as do those of Montcalm. These plants bred true for short awns in the X_3.

(h) Bushy Awned

These plants were observed in the X-ray, Co^{60} low, and Co^{60} high treatments. The heads were almost completely sterile, the florets being composed of a series of hulls progressively surrounding each other to the main outer hull of the floret. The heads thus had the appearance of being fertile but only very few seeds were found. Each floret had 3 to 10 awns extending from it thus giving the plants a bushy appearance (See Fig. 5, Nos. 7, 8, 9 and 10).

(i) Waxy

Several plants in one X_2 tiller progeny row from the betatron treatment had a very shiny, waxy appearance. These plants bred true for the waxy appearance in the X_3,

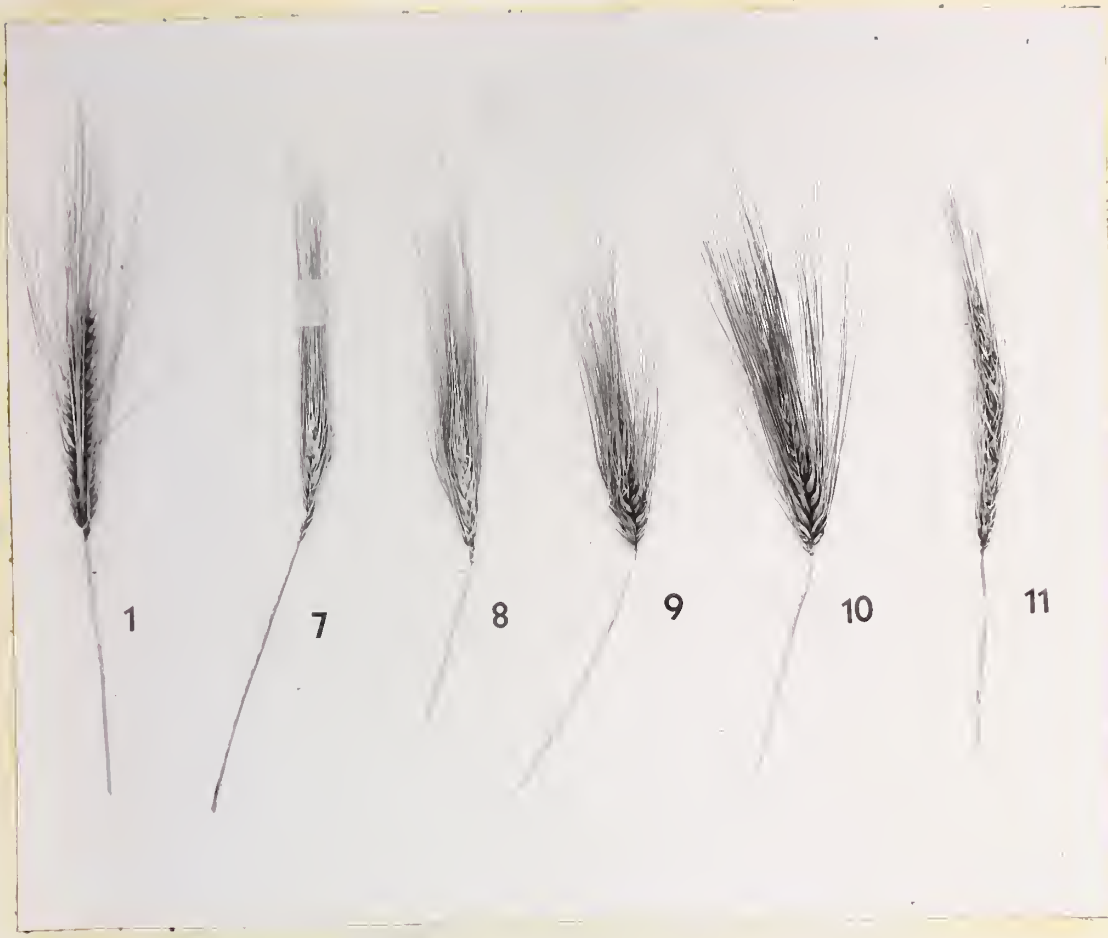

Fig. 5 1, Montcalm; 7, 8, 9, 10, Bushy awned; 11, Curly laterals

(j) Multiflorous

A multiflorous plant was observed in the betatron treated material. It had supernumery florets giving a somewhat bushy appearance to the heads. This mutant bred true in the X_3.

(k) White Heads

This mutant appeared to be identical with Montcalm until heading out, at which time it was noted that the heads were white. This whiteness was evident until the ripened heads became weathered. This mutant differed from grandpa in that only its heads

were white and in that it was the same height as Montcalm. Grandpa, on the other hand, invariably had a white culm, usually variegated leaves, and was somewhat shorter than the Montcalm checks. The white head mutant was not noted in the X_3.

(1) Absent Lower Laterals

This mutant occurred in the Co^{60} low treatment. It was characterized by a lack of lateral florets on the basal part of the head. The plants bred true for this character in the X_3. (See Fig. 6)

Fig. 6 Absent lower laterals

(m) Curly Laterals

Another mutant occurred in this treatment (Co^{60} low) on which the awns of the lateral florets were curly or wavy. This bred true in the X_3. (See Fig. 5, No. 11)

(n) Crooked Neck

In the X-ray, Co^{60} low, and Co^{60} high treatments mutants were observed which had crooked necks. (See Fig. 7, Nos. 12, 13, 14 and Fig. 8, No. 15). This character bred true in the X_3. The type shown in Fig. 8, No. 15 produced plants in the X_3 which were very broad leaved, late, and which in most cases failed to head. Those which did head out failed to set seed before being killed by fall frosts.

(o) Narrow Leaved

The radium-beryllium treatment gave rise to a mutant having slim, narrow leaves. Unfortunately this mutant could not be observed in the X_3 due to accidental damage.

(p) Branched Head

One plant with a branched head was observed in the X_2 of the X-ray treated material. This plant produced only normal plants in the X_3. (See Fig. 8, No. 16).

(q) Dwarf Plants

In the summer of 1953 numerous dwarf plants were noted in all treatments and also in the check materials. (See Fig. 9). These dwarf plants were also observed in plots of other barley varieties such as Gateway. A number of the X_2 dwarf plants from both the treated

material bred true for the dwarf characteristics, some from the treated material and all from the checks appeared normal in the X_3. The number of dwarf plants observed in the nursery in the X_2 was so numerous that they could not all be checked in the X_3, so differences in the number of dwarf plants from the different treatments could not be ascertained.

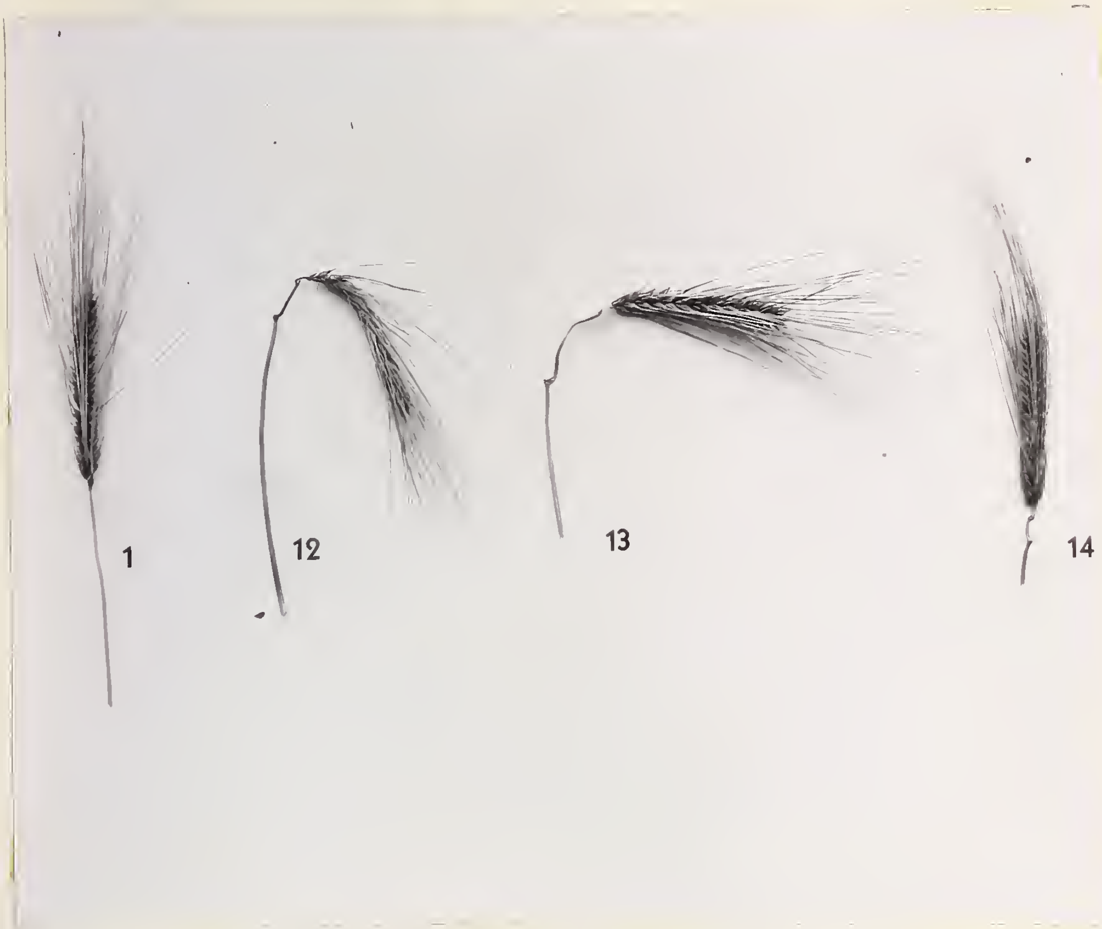

Fig. 7 1, Montcalm; 12, 13, 14, Crooked neck.

Fig. 8 1, Montcalm; 15, Crooked neck; 16, Branched head.

Fig. 9 Left, Montcalm; Right, Dwarf.

In addition to the numerous mutants listed above, a considerable number of sterile plants was found throughout the entire nursery.

C. Observed Effects of Treatments in X_3

Work on this generation was confined, first, to studies of segregation among X_3 plants derived from selected X_2 head rows that had shown mutations and, secondly, to increasing the seed of mutations of possible agricultural value with a view to further testing.

The results on mode of inheritance for the mutants tested are given in Table III.

Table III. Segregation in the X_3 generation, with X^2 tests for goodness of fit to the 3:1 ratio.

Type of mutant	Separate mutations, no.	Normal plants no.	Mutant plants, no.	X^2	P
Albinos	221	690	225	.08	.95
Yellows	63	812	257	.62	.50
Orange	5	500	184	1.32	.30
Yellow-green	76	2526	832	.09	.95
White, green-tipped	15	284	85	.77	.30
Yellow, green-tipped	7	709	264	2.36	.10
Variegated	13	419	134	.18	.95
Mottled	2	159	54	.01	.95
Dense headed	6	551	174	.39	.50
Hooded	1	52	15	.25	.50
Spindly	1	91	35	.56	.50
Bushy awned	5	123	32	1.57	.20

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D. Mutation Rates Relative to Source of Radiation

A summary of the distribution per treatment of the plant progenies which showed chlorophyll aberrants or vital mutations is presented in Table IV.

Gustafsson and Mac Key (20) reported that after irradiation with 10,000 r units of X-ray about 500 chlorophyll aberrants could be expected from the progeny of 10,000 X_1 spikes or, about five per cent. The aggregate results for all five treatments used in this study were in fairly close agreement with this expectation, but the results from the betatron (4.3 per cent) and from the Co^{60} high (3.7 per cent) treatments were somewhat lower.

The results for the betatron treatment in this experiment (4.3 per cent) are strikingly different from those obtained in an earlier study (15.2 per cent) (29). This result is probably due to the method of treatment. In the earlier study a phantom made of pressed wood was inserted between the seed being irradiated and the source. This phantom increased the density of ionization at the level of the kernels. In the present study no phantom was used.

Results from the two Co^{60} treatments indicate that the intensity of treatment has an effect on the mutation rate. The Co^{60} low treatment was made with a source having a dose-rate of 4.5 r per minute at the level of the seeds and gave 5.3 per cent chlorophyll mutants and 1.2 per cent vital mutants. The Co^{60} high treatment was done with a source having a dose-rate of 75.75 r per minute at the

level of the seeds and gave 3.7 per cent chlorophyll mutants and .4 per cent vital mutants. This suggests a need for a more extensive study of this phase.

Table IV. Rates at which chlorophyll-deficient and vital mutants were produced in relation to source of radiation.

Source	Level of treatment	Plant progenies grown, no.	Progenies with chlorophyll deficiencies, no.	Progenies with vital mutants, no.	Chlorophyll deficiencies, %	Vital mutants, %
Betatron	10,000r	1708	73	13	4.3	.8
X-ray	10,000r	1765	103	19	5.8	1.1
Radium-beryllium	10,000r	1355	76	14	5.6	1.0
Co^{60} low	10,000r	1849	99	22	5.3	1.2
Co^{60} high	10,000r	1420	53	6	3.7	.4
Checks		1581	3	0	.2	

VI DISCUSSION

The first objective of this project was to produce useful mutants in Montcalm barley. Stiff-strawed and early mutants, which may be of considerable agricultural value have been obtained, but final assessment of their value will have to await further tests for yield and quality. Only these final tests can give the answer as to whether or not this objective has been successfully achieved. In any event, mutations have been obtained that are superior to the

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[illegible]	[illegible]	[illegible]	[illegible]	(8)	[illegible]	[illegible]	[illegible]
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mother line in certain aspects and, although they may not be directly useful as new varieties, they may well serve as parental material in a hybridization program.

The second objective was to evaluate the findings of Swedish workers and confirm results previously obtained at the University of Saskatchewan. This has been generally achieved, especially through the obtaining of stiff-strawed and early mutants. These results show that the induction of certain types of mutations by irradiation of plant material is reproducable indicating that the radiation technique can be used on any suitable variety or strain with good possibilities of obtaining economically important characters such as stiff straw and earliness.

The third objective was to obtain a comparison of the mutagenic value of various radiation sources. As shown in Table IV, none of the other radiation sources used was better than the X-ray machine relative to the number of mutations induced. However, the betatron and Co^{60} high treatments appear to be somewhat less effective than X-rays in this respect. These results may be explained by differences in the density of ionization. If phantoms had been used when making the betatron and Co^{60} high treatments the results might have been quite different. The X-ray treatment appeared to be most effective in producing the erectoid type of mutation. Three of these mutants were found in the X-ray treatment, none in the Co^{60} low and one in each of the other three treatments. The X-ray treatment also gave a slightly greater range in mutant types than was found in the materials of the other treatments.

The fourth objective was to attempt an evaluation of the induced-mutation technique as a method of plant breeding. In considering this question and in making a comparison between mutation induction techniques and other plant breeding methods, one must consider the time and costs involved and the results achieved.

When one considers induced mutations in relation to the three conventional methods of plant breeding: introduction, selection, and hybridization, it does perhaps at first appear as if it were of doubtful value. But when one considers the limitations of these methods, the possibilities of the induced-mutation technique appears more promising. Introduction as a method of plant breeding is limited by the degree to which available exotic types may be adapted to a plant breeders needs. Selection as a method of plant breeding is limited by the observational powers of the breeder and by the amount of variability of the crop. Hybridization as a method is limited by the quality of available parents and by the number of crosses that can be handled. The induced-mutation technique would also be limited by the amount of material that could be handled, but would not be limited by the amount of variability in the mother lines, nor by serious questions of adaptation to local conditions. Mutation induction might be used to produce new variability which would project the scope of selection and hybridization work well beyond the present limits of these methods.

The time element is a very important factor in plant breeding,

and in this connection the induced-mutation technique may have a marked advantage over hybridization. Mutations are very preponderantly recessive. When a recessive mutant of direct agronomic value appears in the X_2, it has the purity of the mother line and may be increased without further selection. In a hybridization program many generations of hybrids and often much selection work are necessary to attain comparable purity.

In the relatively short period of 27 years induced-mutation is rapidly becoming recognized as a new technique for plant breeding. Two years after the initiation of the present project 16 mutant lines are ready to be entered into yield tests. On the basis of these results it is concluded that induced-mutations should have a place in a plant breeding program and should no longer be neglected for this purpose.

VII SUMMARY

This project was stimulated by Swedish reports that so-called "vital mutants", produced by X-irradiation, may have possibilities either as new commercial barley varieties or as good parental materials.

Four sources in addition to an X-ray machine were used to induce heritable changes in Montcalm barley. All treatments were made at the 10,000 r level. The sources with their respective dose-rates in the region of the irradiated seeds were as follows:

1. An X-ray machine operated at 200 K.V.P. and 20 m.a. with no filter and a dose-rate of 201 r per min.

2. A betatron operating at 24 M.E.V. with a dose-rate of 181.8 r per min.

3. A radium-beryllium source with a dose-rate of 5.3 r per min.

4. A Co^{60} low source with a dose-rate of 4.5 r per min.

5. A Co^{60} high source with a dose-rate of 75.75 r per min.

Results indicate that the storage of seeds after treatment may reduce their ability to survive irradiation treatments when sown at a later date.

None of the irradiation sources used was better than the X-ray treatment in relative mutation rate. The betatron and Co^{60} high treatments appear to be somewhat less effective than X-rays in this regard.

The mutants observed in this project are listed as follows:

Albinos;	Hooded;
Yellows;	Singed;
Orange;	Leafless;
Yellow-green;	Spindly or vine-like;
White, green-tipped;	Tweaky;
Yellow, green-tipped;	Short awns;
Variegated or striped;	Bushy awned;
Mottled;	Waxy;
Grandpa;	Multiflorous;
Stiff-strawed;	White heads;

Dense headed;

Weak strawed;

Early maturing;

Absent lower laterals;

Curly laterals;

Crooked neck.

In addition to these mutants narrow-leaved, branched-head, dwarf, and sterile mutants were observed. All mutants were recessive and segregated in the ratio of 3 normal: 1 mutant in the X_2 generation.

A definite conclusion on the usefulness of induced mutations in plant breeding must await further testing of the mutants obtained. However, on the basis of the results obtained and on the reports of other workers it is felt that induced mutations have a definite place as a new approach in plant breeding.

VIII ACKNOWLEDGEMENTS

The writer wishes to express his appreciation to Mr. R.W. Hummel and Dr. J.W.T. Spinks of the Chemistry Department, University of Saskatchewan, and Dr. H.E. Johns of the Physics Department, University of Saskatchewan for making the radiation sources available and for performing the treatments. Thanks are also extended to the Field Husbandry Department, University of Saskatchewan, Plant Science Department, University of Alberta, and to Mr. G.N. Denike, Superintendent, Dominion Experimental Station, Swift Current, Saskatchewan for generously providing facilities to make the project possible. The writer gratefully acknowledges his indebtedness to Dr. L.P.V. Johnson, Professor of Genetics and Plant Breeding, for his assistance, guidance and advice during the course of the study. Grateful acknowledgement is also made to the Barley

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Improvement Institute for its financial support to the project.

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